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Imbalance between tumour necrosis factor-alpha and soluble TNF receptor concentrations in severe meningococcaemia

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SUMMARY

The extracellular domain of tumour necrosis factor-alpha (TNF-α) receptors have inhibitory properties against TNF-α. The relative ratio between ligand and ligand inhibitors may influence the outcome of meningococcaemia. To test this hypothesis, levels of TNF-α and of each of the soluble inhibitory fragments originating from two distinct TNF-α receptors (sTNF-RI and sTNF-RII) were measured in sera of children with severe meningococcaemia. On admission to the hospital the levels of sTNF-RI, -RII and TNF-α were markedly increased and all three correlated with the outcome of the disease. A correlation was found between TNF-α and sTNF-RI ($P < 0.001$ by Pearson rank correlation coefficient) or sTNF-RII ($P = 0.012$). For TNF-α concentrations below 500 pg/ml, the increase of TNF-α was proportional to that of sTNF-RI and RII; however, when TNF-α levels exceeded 500 pg/ml, sTNF-RI and RII concentrations did not increase proportionally. At admission, in patients with fatal outcome, the ratios TNF-α/sTNF-RI and -RII were higher than in survivors. During the first 6 hr, the kinetics of TNF-α, sTNF-RI and -RII were different. Naturally occurring TNF-α inhibitors may play an important role in modulating the biological activity of TNF-α in severe meningococcaemia.

INTRODUCTION

Tumour necrosis factor-alpha (TNF-α) is an important mediator of inflammation. At low concentrations, TNF-α contributes to homeostasis and host defence mechanisms, whereas at high concentrations it mediates various severe pathological conditions, particularly haemodynamic changes observed in Gram-negative septic shock.[1,2] Serum TNF-α concentrations correlate with the severity of infectious purpura and meningococcal infections.[3,4]

TNF-α could be easily measured in sera in pathological conditions but its true biological activity has been debated.[5] In fact, human urine and serum, particularly in pathological conditions, contain proteins which can interfere with the functions of TNF-α when tested in assays of cytotoxicity, using TNF-α-susceptible cell lines.[6,7] Characterization of these inhibitors revealed two immunologically distinct TNF-α-binding proteins (TBP I and TBP II).[8,9] The same structures were found to be part of the extra-membranous fragments of two TNF-α receptors with apparent molecular masses of 55,000–60,000 MW and 75,000–80,000 MW, respectively.[9,12] The two fragments are also referred to as soluble TNF receptor type I (sTNF-RI) or sTNF-Rβ, and soluble TNF receptor type II (sTNF-RII) or sTNF-Rα.[13–20] Both molecules, TBP and TNF-R are derived from the same transcripts with microheterogeneity at the proteolytic cleavage site. The two receptor fragments, sTNF-RI and sTNF-RII, can be detected in biological fluids by specific monoclonal antibodies. In view of the deleterious effect of TNF-α in septic shock, the relative ratio between TNF-α and its inhibitors may determine the clinical outcome. The purpose of this study was to investigate the relationship of TNF-α with sTNF-RI and -RII in serum of children with severe meningococcaemia at their admission to the hospital and 6 hr later.

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MATERIALS AND METHODS

A biological score was used to depict the severity of the disease:[3] one point was given for each of the following biochemical abnormalities known to be associated with fatal outcome: blood leucocyte count below 10.0×10^9/l, blood platelet count below 100.0×10^9/l, fibrinogen level below 1·5 g/l, serum carbon dioxide level below 15 mM/l and cerebrospinal fluid count below 0.1×10^9/l. The criteria of admission to the study have been described previously.[3] Briefly, children eligible for inclusion were those with a clinical diagnosis of sepsis with purpuric lesions. They were enrolled if they were in shock or if they had three or more of the above biological abnormalities. At

admission to the hospital and after 6 hr of evolution blood was obtained for determination of the biological risk factors, bacterial cultures, serum TNF-α, serum interleukin-6 (IL-6) levels and TNF receptor concentrations. Twenty-one patients had *Neisseria meningitidis* isolated from blood cultures and/or cerebrospiral fluid cultures. Fifteen patients received a dose of antibiotics before or during the transport to the hospital as soon as the diagnosis of meningococcaemia was made.

TNF-α was measured by radioimmunossay (Medgenix Ltd, Fleurus, Belgium), IL-6 was measured on IL-6-dependent murine hybridoma cells 7TD1.[21] sTNF-RI and -RII were assayed by enzyme-linked immunological binding assay according to the method of M. Brockhaus and H. Gallati (manuscript in preparation). Briefly, 96-well microtitre plates were coated with monoclonal antibodies to sTNF-RI (clone htr-20) or to sTNF-RII (clone utr-4), then saturated with bovine serum albumin (BSA) (Sigma Chemical Co, St Louis, MO). Microtitre plates were washed and and 100 μl of standard (human recombinant sTNF-RI and II, provided by Hoffmann La Roche, Basel, Switzerland) or diluted samples were dispensed onto the plates. Peroxidase-conjugated recombinant human TNF-α (Hoffmann La Roche) was added to the wells, and plates were incubated overnight at room temperature. After washing, tetramethylbenzidine was added and incubated for 15–30 min. The reaction was stopped with H_2SO_4 and read at 450 nm. The concentrations of sTNF-RI and sTNF-RII in the samples were determined by interpolation from the standard curve.

Statistics

Continuous values were compared by Mann–Whitney test or by Wilcoxon test for paired data. The slope of the correlation between ligand and ligand inhibitor was analysed using a locally weighted smoothing model.[22] Correlations were tested using Pearson's rank correlation coefficient. Values are given as mean ±SEM. All *P* values are two-tailed.

Table 1. Relation between clinical and biological parameters on admission and outcome

Parameters	Survival	Death	*P**
Age (years)	4·9±0·9 (26)	4·7±1·5 (9)	0·925
Duration of disease (hr)†	19·9±5·1 (26)	15·6±3·3 (9)	0·910
Leucocytes (10^4/l)	10·4±2·1 (25)	5·8±1·3 (9)	0·412
Fibrinogen (g/l)	2·7±0·3 (26)	0·5±0·1 (8)	<0·001
Platelets (10^4/l)	165±28 (25)	39±8 (9)	0·001
TNF-α (pg/ml)	468±80 (26)	1466±285 (9)	0·001
IL-6 (ng/ml)	22·4±5·4 (26)	216·3±84·2 (8)	<0·001
sTNF-RI (ng/ml)	26·3±2·1 (22)	35·4±3·0 (9)	0·007
sTNF-RII (ng/ml)	67·8±7·2 (19)	93·3±8·1 (9)	0·041

* By Mann–Whitney test.

† Intervals between first symptoms and admission to the hospital. Data are mean ±SEM; number of patients in parentheses.

RESULTS

Serum sTNF-RI concentrations were 2·19±0·10 ng/ml and sTNF-RII concentrations were 0·79±0·10 ng/ml in a control group of healthy individuals ($n=9$). High sTNF-RI and sTNF-RII levels were found in the patients with meningococcal sepsis (29·8±1·8 and 76·0±5·9 ng/ml respectively). Clinical and biological parameters obtained at admission are shown in Table 1, classified according to the outcome. The overall mortality rate was 26%. Fibrinogen concentration, platelet count, TNF-α and IL-6 levels were the most efficient predictors of a fatal outcome. Both sTNF-RI and sTNF-RII serum levels were higher in patients with fatal outcome than in survivors.

On admission, a significant correlation was observed, using Pearson's rank correlation coefficient, between the levels of TNF-α and sTNF-RI ($P<0·001$) or sTNF-RII ($P=0·012$). These relations were analysed using a locally weighted smoothing model[22] which revealed two distinct linear relationships (Fig. 1). For TNF-α concentrations below 500 pg/ml, the relation was steep with small changes in TNF-α leading to important increases in shTNF-RI levels ($y=4·84+79·01\ x$). The relation

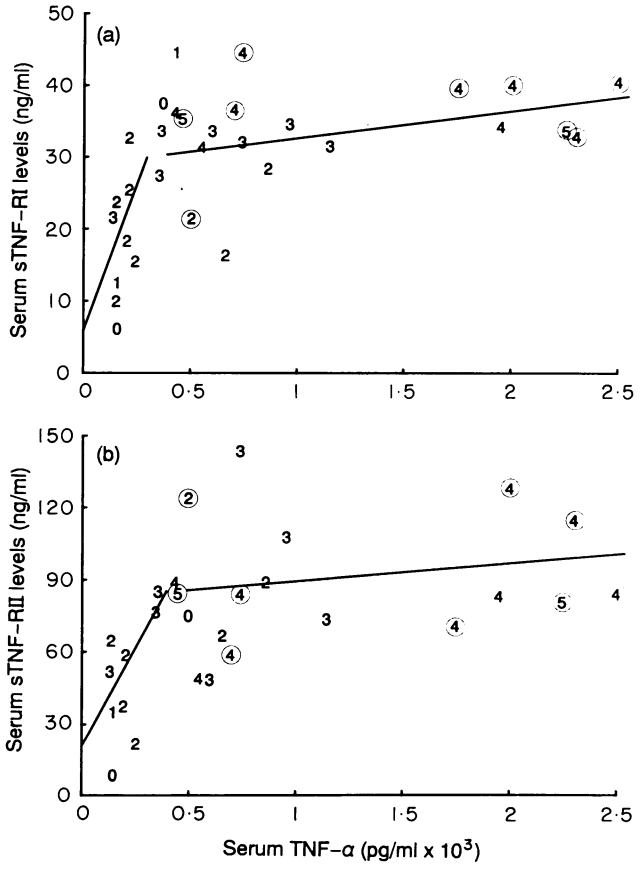

Figure 1. Correlation between TNF-α and sTNF-RI or sTNF-RII. Patients are represented in the correlation by the number of their biological risk factors (as described in Materials and Methods) they had on admission to the hospital. Circled numbers represent patients who died. All the deaths occurred between 10 and 48 hr after admission.

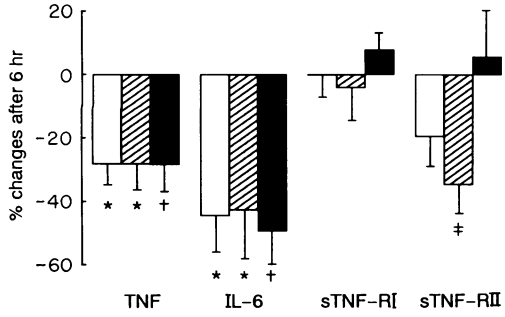

Figure 2. Evolution of the serum concentrations of TNF-α, IL-6, sTNF-RI and sTNF-RII. Values are expressed as per cent of changes between concentrations obtained at admission and after 6 hr of evolution. Data are mean ±SEM. * $P \leq 0.001$, † $P < 0.05$; ‡ $P < 0.01$. Whole group (□); survival (▨); death (■).

Table 2. Evolution of the ratio TNF-α/sTNF-RI and -RII in children with meningococcaemia

	Survival		Death	
Ratio	Admission	6 hr	Admission	6 hr
TNF-α/sTNF-RI × 100	1·8±0·3 ($n=22$)	1·7±0·3 ($n=13$)	4·0±0·8* ($n=9$)	2·1±0·4 ($n=6$)
TNF-α/sTNF-RII × 100	0·8±0·1 ($n=19$)	1·3±0·3 ($n=10$)	1·6±0·3** ($n=9$)	1·0±0·1 ($n=6$)

In the group of patients who died, the drop of the ratio during the 6 hr following admission was significant (Wilcoxon test, $P=0.028$ for TNF-α/sTNF-RI and 0·046 for TNF-α/sTNF-RII).

* $P=0.005$; ** $P=0.029$, compared to values of survivors at admission by Mann-Whitney test.

flattened for TNF-α concentrations >500 pg/ml ($y=28.50+4.08\,x$) (Fig. 1a). The difference between the slopes was highly significant ($P<0.001$). Similar relations were observed with sTNF-RII as shown in Fig. 1b ($y=18.25+145.08\,x$ and $y=79.9+7.25\,x$; $P<0.001$). The slope of the relationship persisted after measuring the sTNF-R at different dilutions in the immunological binding assay (data not shown). Patients are represented by the number of their biological risk factors determined on admission as defined in Materials and Methods. Circles represent patients who died.

The serum concentrations of sTNF-RI, sTNF-RII, TNF-α and IL-6 were also studied 6 hr after admission (Fig. 2). At this time, a decrease of 28% in TNF-α concentrations was observed in non-surviving patients as well as in those who recovered. Similarly, IL-6 serum levels decreased by 44±11% in the whole group, 49±12% in the deceased patient group and 43±15% in the patients with a favourable outcome. The concentration of sTNF-RI remained stable during the first 6 hr of evolution for the two groups of patients, whereas the evolution of sTNF-RII in patients who recovered (drop of 35±9%) differed from that of the deceased patients (increase of 6±16%) (Fig. 2). Considering the inhibitory effects of the two TNF-α receptors, we wondered whether the ratio TNF-α/sTNF-R would be a better indication of mortality than the receptor concentration alone. Indeed, on admission the ratio was higher in the patients who died (Table 2). Six hours after admission, the ratios of TNF-α/sTNF-RI and -RII were comparable in the group of patients who recovered and in those with poor outcome.

DISCUSSION

The data show that high amounts of sTNF-RI or sTNF-RII are produced in a condition where circulating concentrations of TNF-α are increased, and that high values correlate with fatal outcome.

As the extracellular domain of TNF-α receptors have inhibitory properties against TNF-α, the relative ratio between ligand and ligand inhibitors may be related to the severity of meningococcaemia. Two types of correlations were found between TNF-α and sTNF-RI or sTNF-RII. When TNF-α levels were low (<500 pg/ml) the increase in TNF-α was proportional to that of sTNF-R. However, when TNF-α exceeded 500 pg/ml the values of sTNF-RI or sTNF-RII did not increase proportionally, a fact evident only at the early stage of the disease. The imbalance between TNF-α and sTNF-R at this stage could be of great importance ni the physiopathology of the development of shock, TNF-α prevailing over the two inhibitors. In this respect, one of the most relevant facts is the TNF-α/sTNF-RI ratio at the time of hospital admission as it appears to be of predictive value for a fatal outcome. After 6 hr of evolution, the TNF-α/sTNF-RI ratio was similar in the group of patients who recovered and in those who died.

During these 6 hr a disparity was observed in the evolution of TNF-α and its receptors which could be related to a difference in the kinetics between the ligand and its inhibitors. The concentrations of the two sTNF-R also evolved differently, which may be indicative of a distinct regulation of receptor expression resulting either from increased expression on certain types of cells and/or increased cleavage of the extracellular moiety of the receptor.[10]

Recently, recombinant human TNF receptor fragments have been found to be bioreactive and protective against lethality in a model of D-galactosamine-sensitized mice injected with endotoxin.[23] Naturally occurring TNF-α inhibitors may play an important role in modulating the biological activity of TNF-α when the latter occurs in high levels, as in meningococcaemia. It is tempting to speculate that at a given time a critical imbalance arises between ligand and ligand inhibitors that decides the biological activity of TNF-α and correlates with the clinical outcome.

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